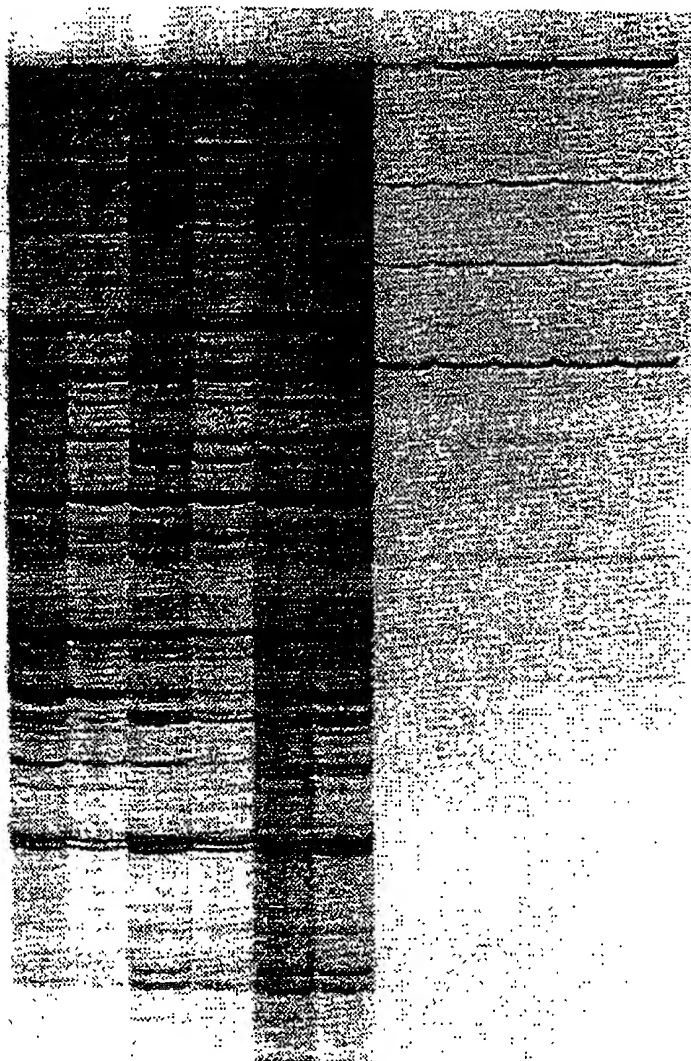


Figure 1A.

# RNA Fingerprinting Studies of MCF-7 Cells

MCF-7		MCF-7		MCF-7		Controls				
W		AdrVp		AdrVpPR						
1	2	3	4	5	6	7	8	9	10	11



099103 0010  
101200 9801960

Figure 1B.

# Northern Blot Hybridization of PCR Product "Clone-8"

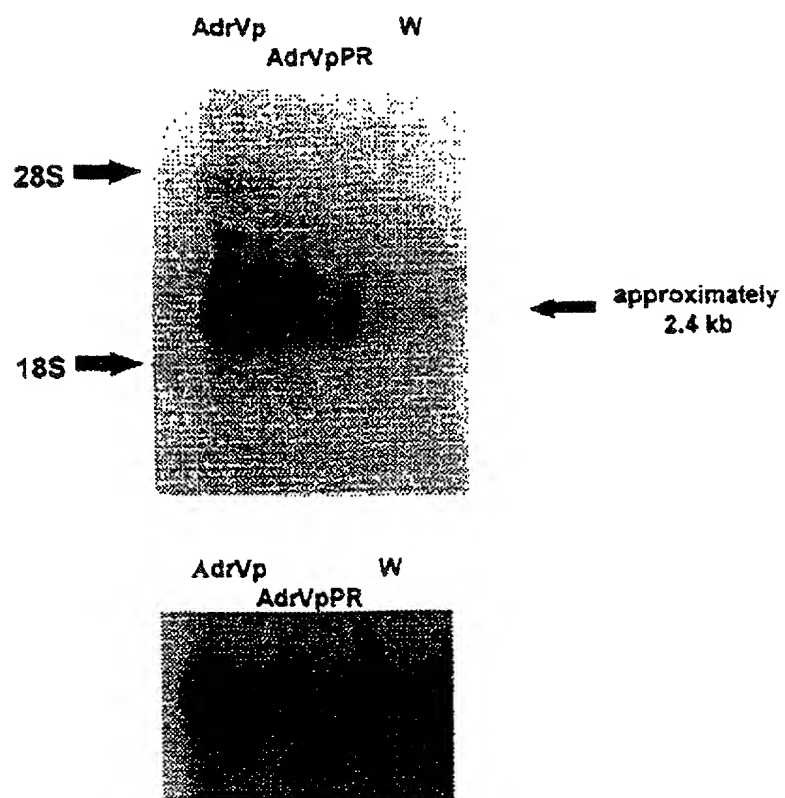
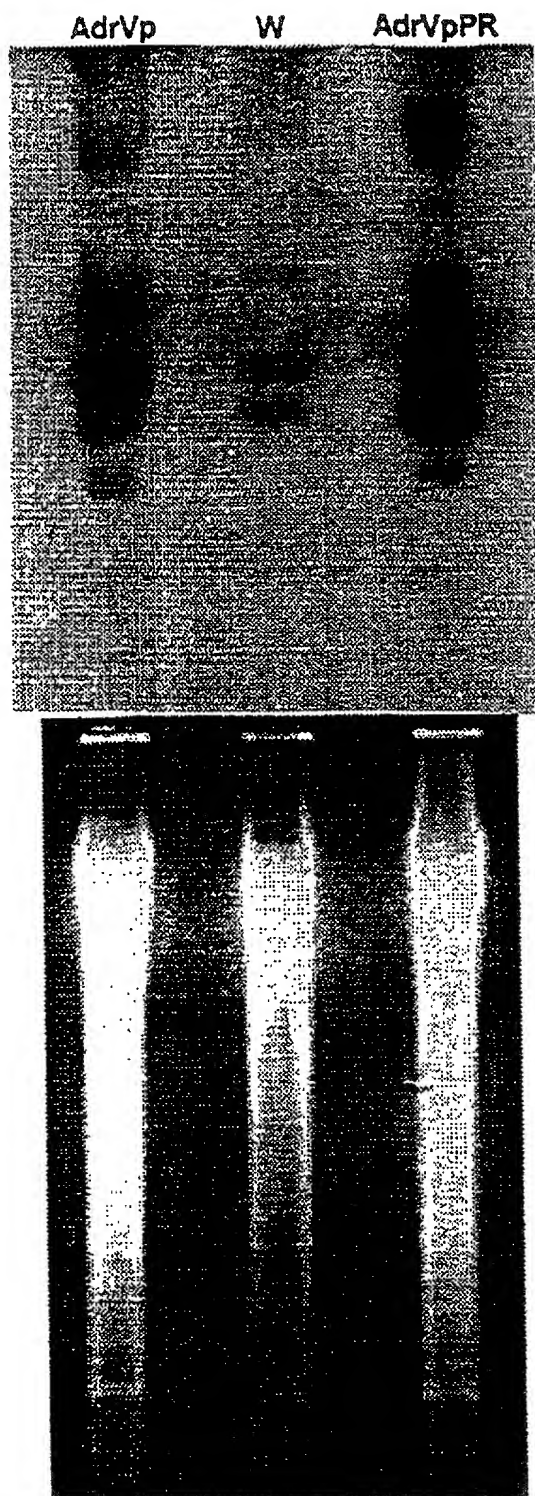


Figure 1C.

# Southern Blot Hybridization--Clone 8




20250909 10:00:00

Figure 2A.

## Peptide Sequence of BCRP


Length: 655 amino acids

1 MSSSNVEVFI PVSQGNNGF PATASNDLKA FTEGAVLSFH NICYRVKLKS


51 GFLPCRKPVE KEILSNINGI MKPGLNAI  SSLL DVAARKDPSG  
Walker A motif

101 GLSGDVLING APRPANFKCN SGYVVQDDVV MGTTLTVRENL QFSAALRLAT



151 TMTNHEKNER INRVIQELGL DKVADSKVGT QFIRGVSGGE RKRTSIGMEL

201 ITDPSILFLD  MSKQGRITIF SIHQPRYSIF  
Phosphopantetheine site

251 KLFDSTLLA SGRLMFHGPA QEALGYFESA GYHCEAYNNP ADFFLDIING




301 DSTAVALNRE EDFKATEIIE PSKQDKPLIE KLAELV  FYKETKAELH  
Glyc

351 QLSGGEKKKK ITVFKEISYT TSFCHQLRWV SKRSEKNLLG NPQASIAQII

401  VTVVLGLVIG AIYFGL  TGIQNRAGVL FELTNQCFS SVSAVELEVV  
TM 1 Glyc

451 EKKLFIHEYI SGYYRVSSYF LSKLLSDLLP MTMLPSIIFT CIVYFMLGLK

501 PKADAFFVMM FTLMVAYSA SSMALAIAG QSVVSVATLL MTICEVFMMI  
TM 2

551  FSGLLV  FCPGL  TGN  
Glyc Glyc

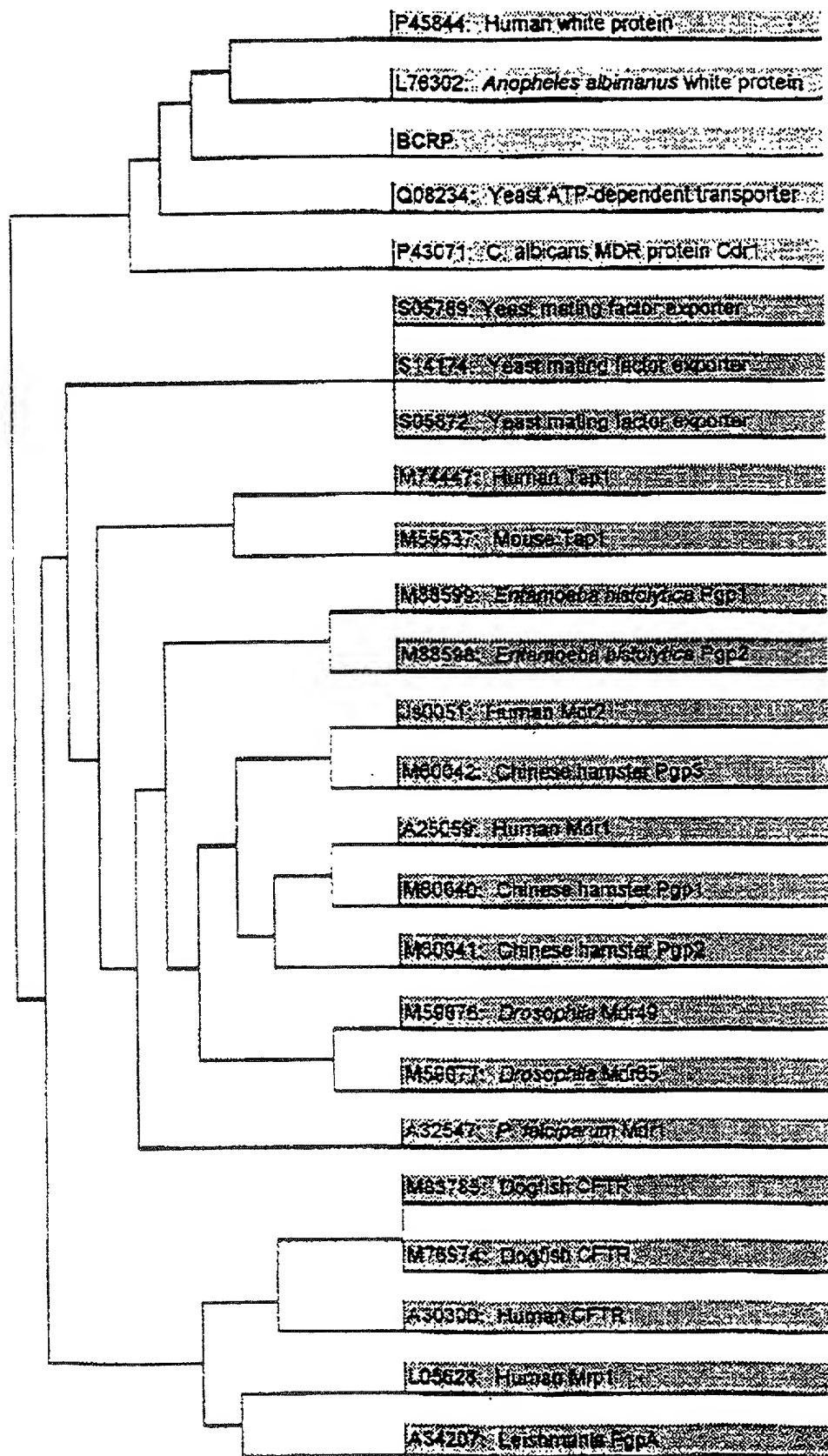
601 NPCNYATCTG EEYLVKQGID LSPWGLWKNH VALACMIVIF LTIAYLKLLE  
TM 3

651 LKKYS

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Figure 2B. Pileup dendr

Relative Similarity of BCRP to Selected Members  
of the ABC Transporter Superfamily



```

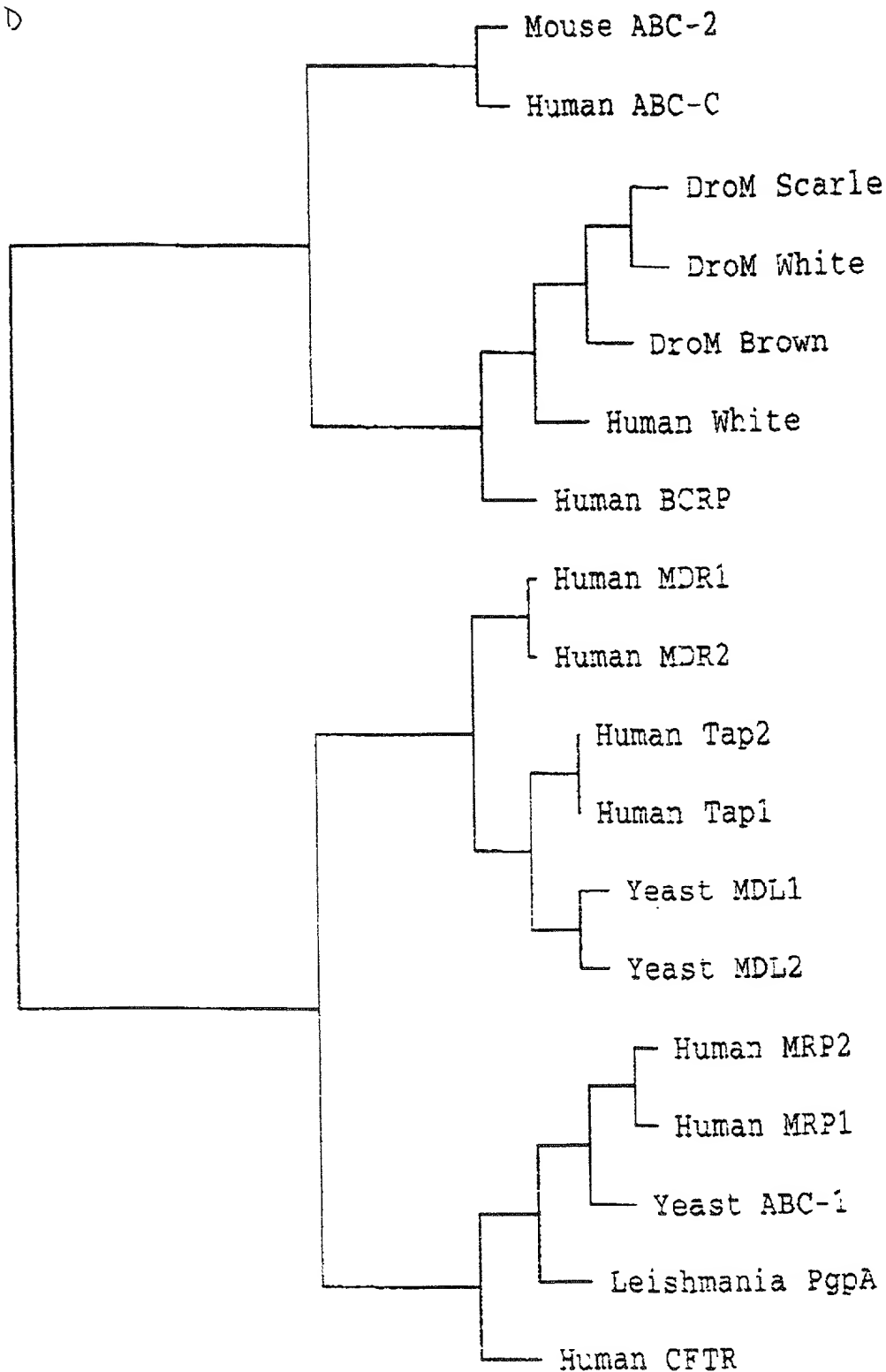
1 GGGAGGAGGC AGCCTGTGGA GGAAGTGGGT AGGATTTAGG AACGCACCGT
51 GCACATGCTT GGTGGTCTTG TTAAGTGGAA ACTGCTGCTT TAGAGTTTGT
101 TTGGAAGGTC CGGGTGACTC ATCCCAACAT TTACATCCTT AATTGTTAAA
151 GCGCTGCCTC CGAGCGCACG CATCCTGAGA TCCTGAGCCT TTGGTTAAGA
201 CCGAGCTCTA TTAAGCTGAA AAGATAAAAA CTCTCCAGAT GTCTTCCAGT
251 AATGTCGAAG TTTTATCCC AGTGTCAACA GGAAACACCA ATGGCTTCCC
301 CGCGACAGCT TCCAATGACC TGAAGGCATT TACTGAAGGA GCTGTGTTAA
351 GTTTTCATAA CATCTGCTAT CGAGTAAAAC TGAAGAGTGG CTTTCTACCT
401 TGTCGAAAAC CAGTTGAGAA AGAAATATTA TCGAATATCA ATGGGATCAT
451 GAAACCTGGT CTCAACGCCA TCCTGGGACC CACAGGTGGA GGCAAATCTT
501 CGTTATTAGA TGTCTTAGCT GCAAGGAAAG ATCCAAGTGG ATTATCTGGA
551 GATGTTCTGA TAAATGGAGC ACCGCGACCT GCCAATTTC AATGTAATTC
601 AGGTTACGTG GTACAAGATG ATGTTGTGAT GGGCACTCTG ACGGTGAGAG
651 AAAACTTACA GTTCTCAGCA GCTCTTCGSC TTGCAACAAC TATGACGAAT
701 CATGAAAAAA ACGAACGGAT TAACAGGGTC ATTCAAGAGT TAGGTCTGGA
751 TAAAGTGGCA GACTCCAAGG TTGGAAGTCA GTTTATCCGT GGTGTGCTCG
801 GAGGAGAAAG AAAAAGGACT AGTATAGGAA TGGAGCTTAT CACTGATCCT
851 TCCATCTTGT TCCTGGATGA GCCTACAAC TGCCTTAGACT CAAGCACAGC
901 AAATGCTGTC CTTTGTCTCC TGAAAAGGAT GTCTAAGCAG GGACGAACAA
951 TCATCTTCTC CATTCATCAG CCTCGATATT CCATCTTCAA GTTGTTTGAT
1001 AGCCTCACCT TATTGGCCTC AGGAAGACTT ATGTTCCACG GGCCTGCTCA
1051 GGAGGCCTTG GGATACTTTG AATCAGCTGG TTATCACTGT GAGGCCTATA
1101 ATAACCCCTG AGACTTCTTC TTGGACATCA TTAATGGAGA TTCCACTGCT
1151 GTGGCATTAA ACAGAGAAGA AGACTTTAAA GCCACAGAGA TCATAGAGCC

```



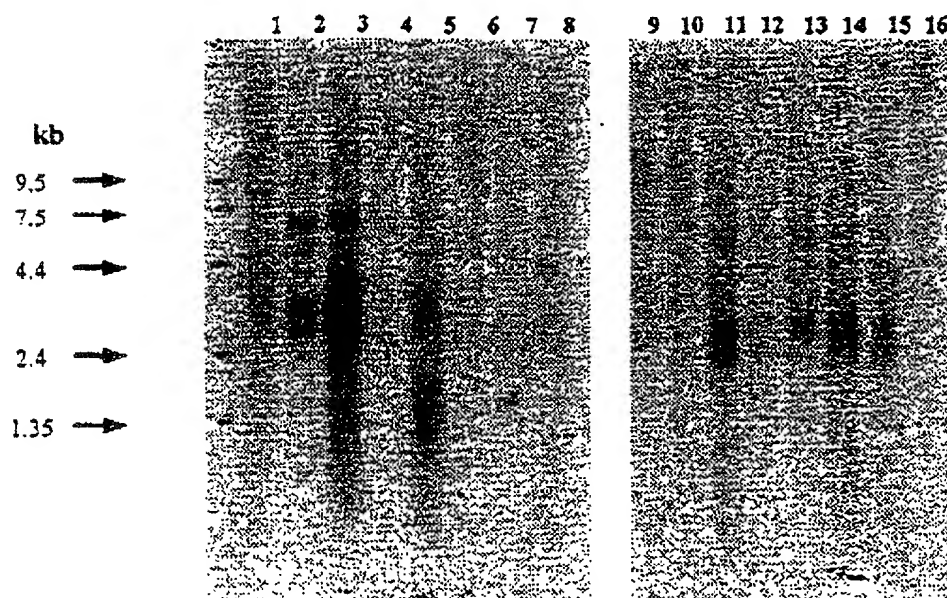
Figure 2D

Growthtree Phylogram of Selected ABC Transporters





### Northern Blot Analysis using Clone 8 cDNA as Probe



**Multiple tissue Northern Blots.** Key to lane numbering is as follows:

- |                    |                                 |
|--------------------|---------------------------------|
| 1. Heart           | 9. Spleen                       |
| 2. Brain           | 10. Thymus                      |
| 3. Placenta        | 11. Prostate                    |
| 4. Lung            | 12. Testis                      |
| 5. Liver           | 13. Ovary                       |
| 6. Skeletal Muscle | 14. Small Intestine             |
| 7. Kidney          | 15. Colon                       |
| 8. Pancreas        | 16. Peripheral blood leukocytes |

Figure 4A.

Expression of BCRP mRNA in Subclones of MCF-7/W cells  
Stably Transfected with the Expression Vector pcDNA3-BCRP

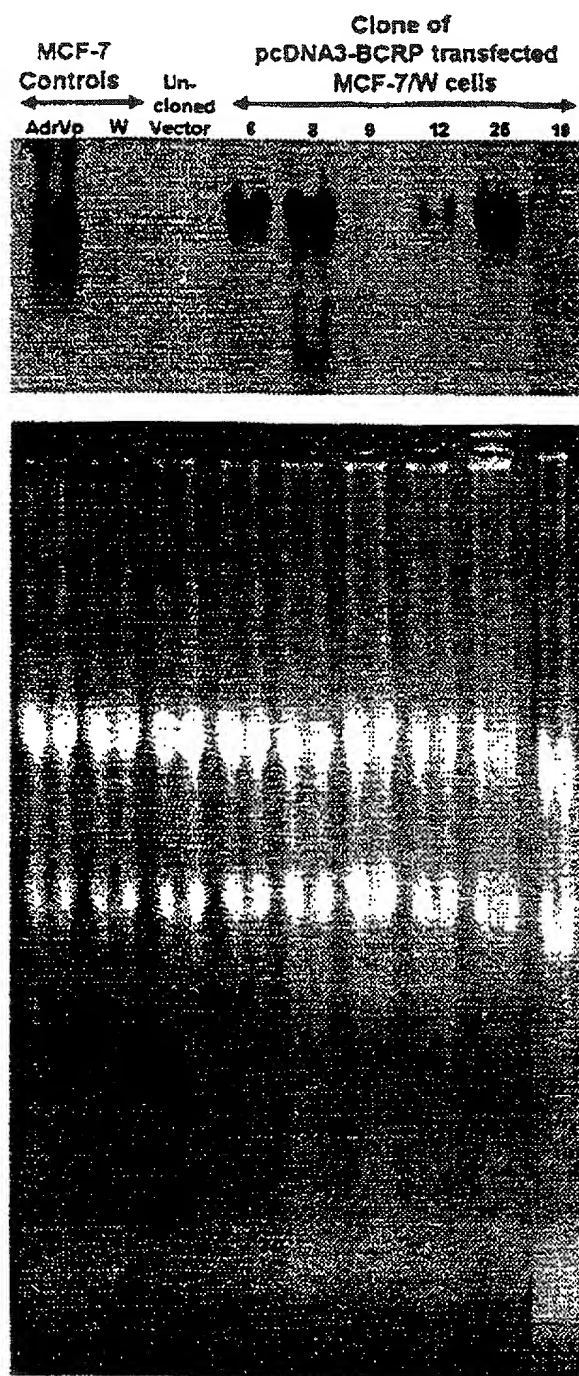


Fig 4B

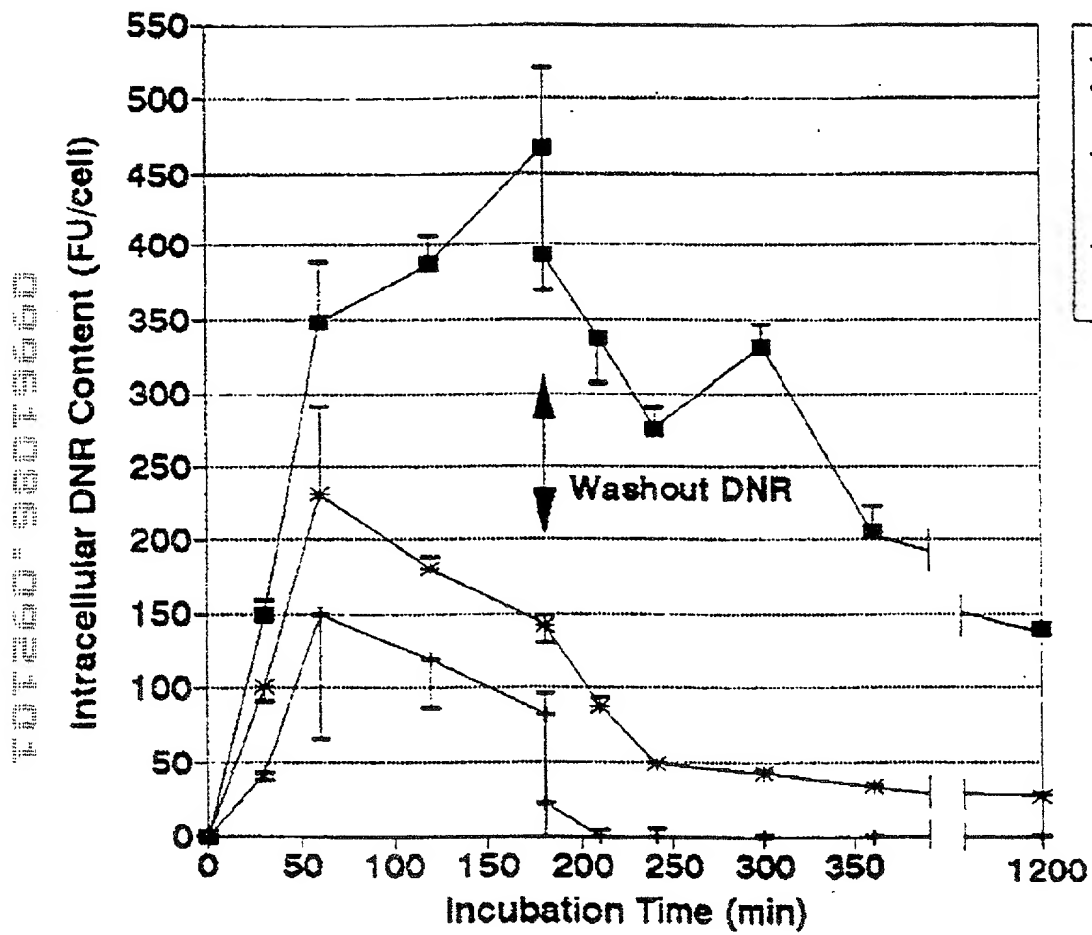


Figure 4C

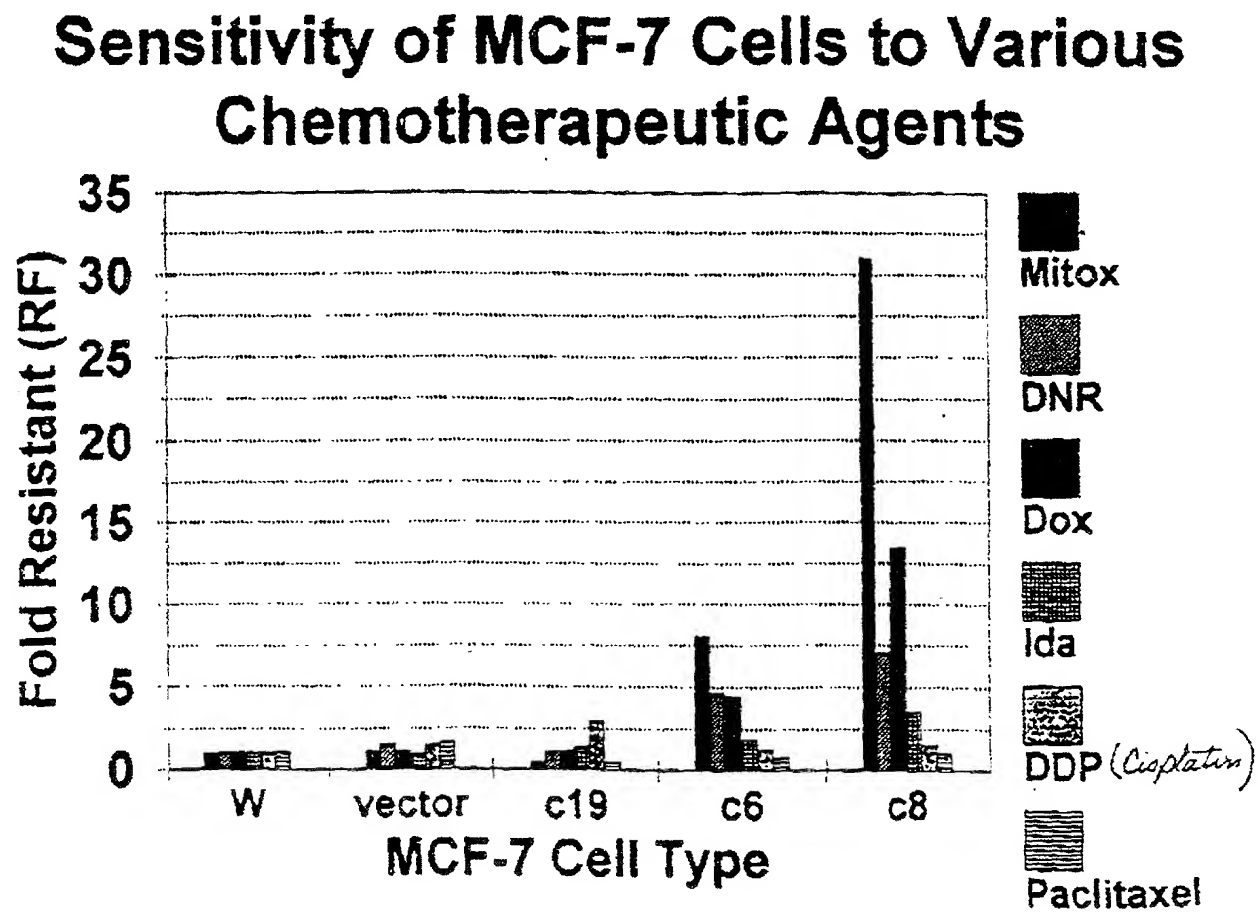
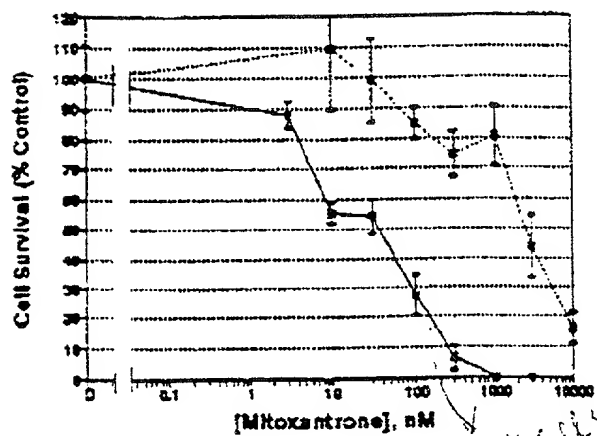


Figure 4D



wt cells

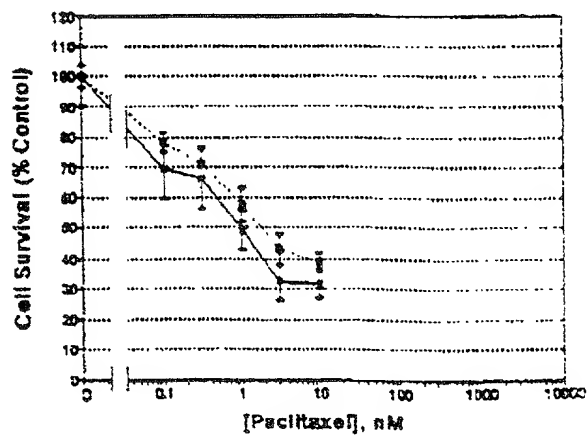
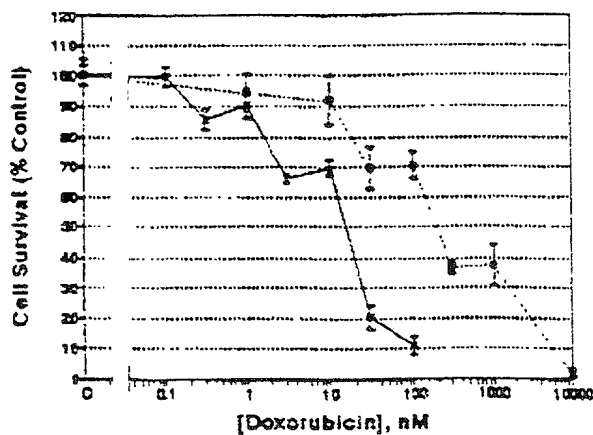
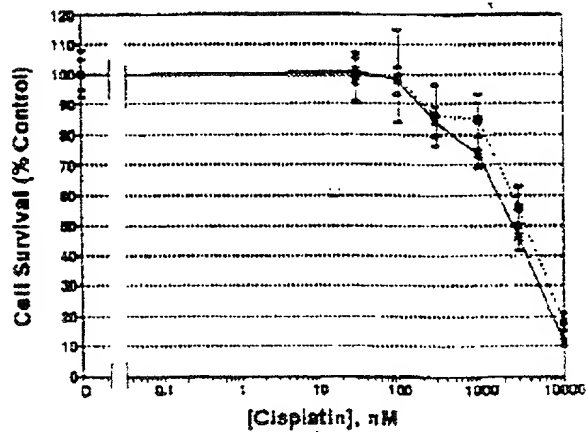
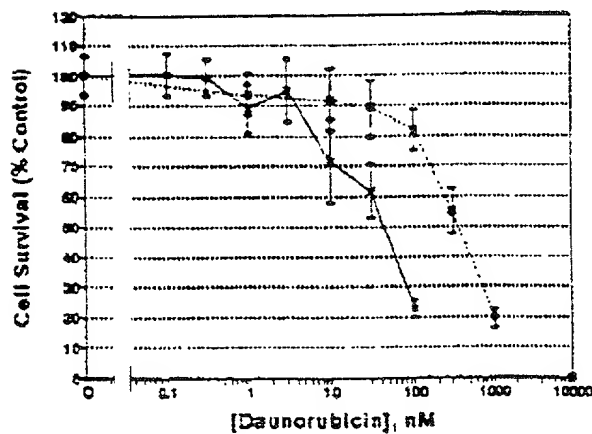
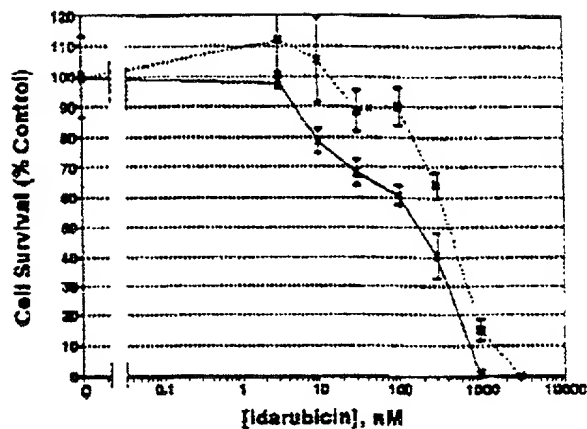
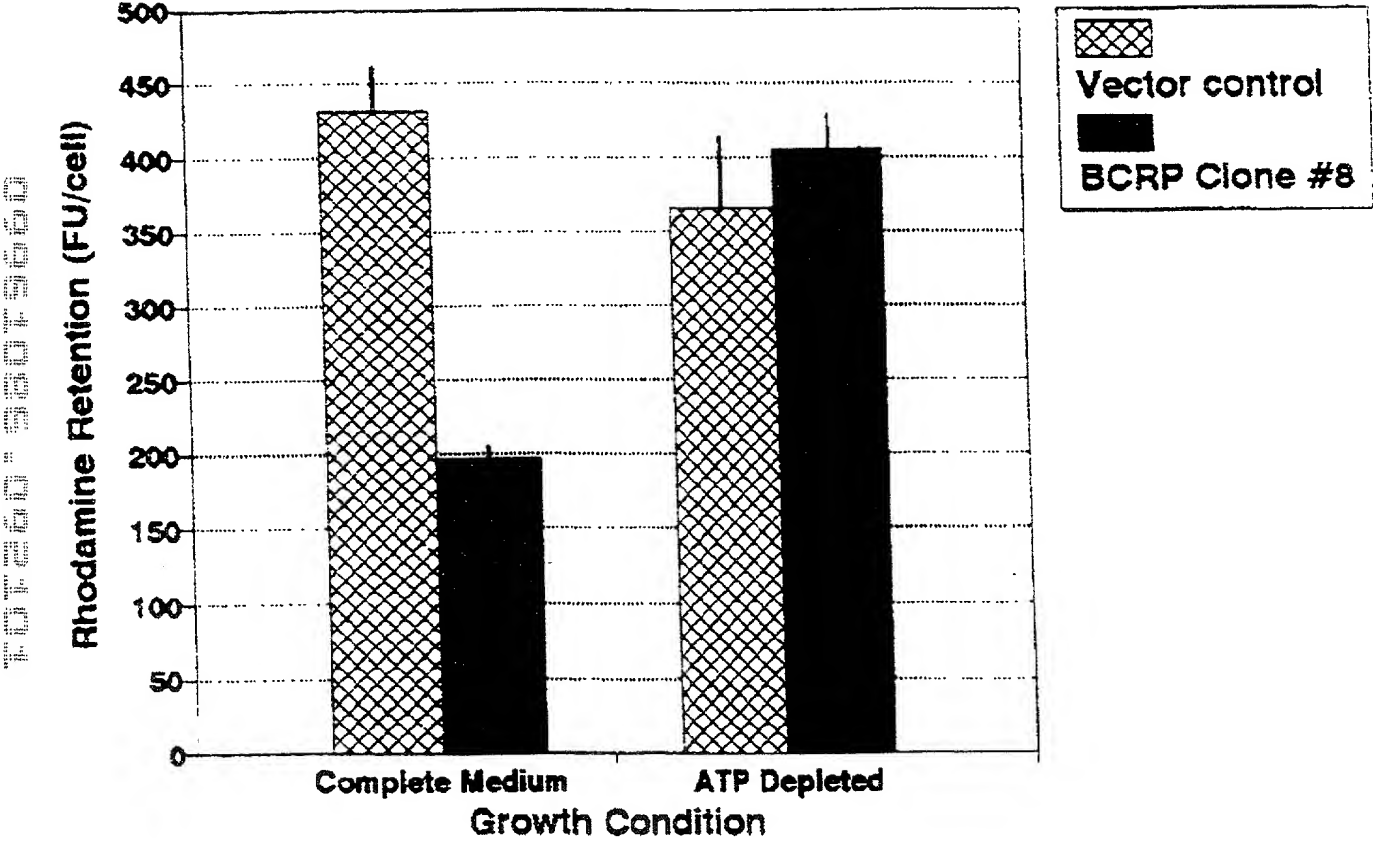


Figure 4C



**Figure 5**  
**Effects of Chemotherapeutic Drugs on BCRP-transfected MCF-7 Cells**

### Effects of Chemotherapeutic Drugs on BCRP-transfected MCF-7 Cells

Cell Line	Mitoxantrone		Daunorubicin		Doxorubicin		Idarubicin		CisPlatin		Paclitaxel	
	LC50	RF	LC50	RF	LC50	RF	LC50	RF	LC50	RF	LC50	RF
MCF-7/W	48	1.0	47	1.0	57	1.0	76	1.0	2,357	1.0	1.9	1.0
MCF-7/pcDNA3	54	1.1	72	1.5	66	1.2	126	1.7	3,525	1.5	3.0	1.6
MCF-7/BCRPe19	21	0.4	54	1.1	67	1.2	107	1.4	6,950	2.8	0.8	0.4
MCF-7/BCRPe6	393 <sup>***</sup>	8.2	218 <sup>***</sup>	4.5	254	5.2	140	1.8	3,080	1.3	1.4	0.7
MCF-7/BCRPe8	1,496 <sup>***</sup>	31.2	328 <sup>***</sup>	7.0	768 <sup>*</sup>	9.2	265	3.5	3,700	1.6	1.8	0.9
MCF-7/AdrVp	160,000 <sup>***</sup>	3333	1667 <sup>***</sup>	35.5	8650 <sup>***</sup>	175.0	70	0.9	4,700	2.01	2.8	1.5

<sup>a</sup> = differs significantly from MCF-7/W or MCF-7/pcDNA3, *p* < 0.05 (Student's *t* test)

\* = differs significantly from MCF-7/V or MCF-7/pcDNA3,  $p < 0.01$  (Student's *t* test)

[illegible]

Figure 6

Expression of Human w gene in MCF-7 Cells,  
Detected by RT-PCR

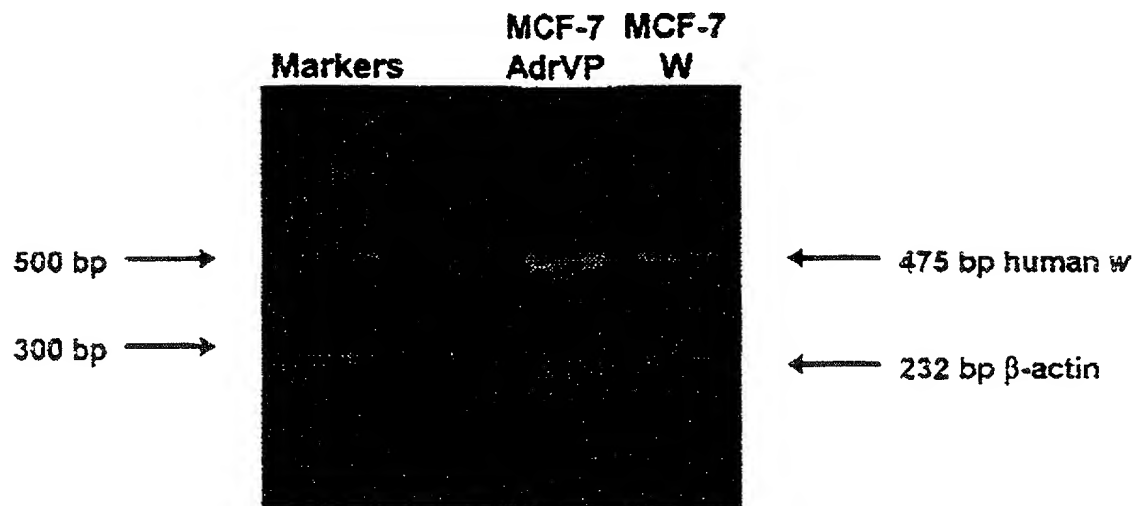
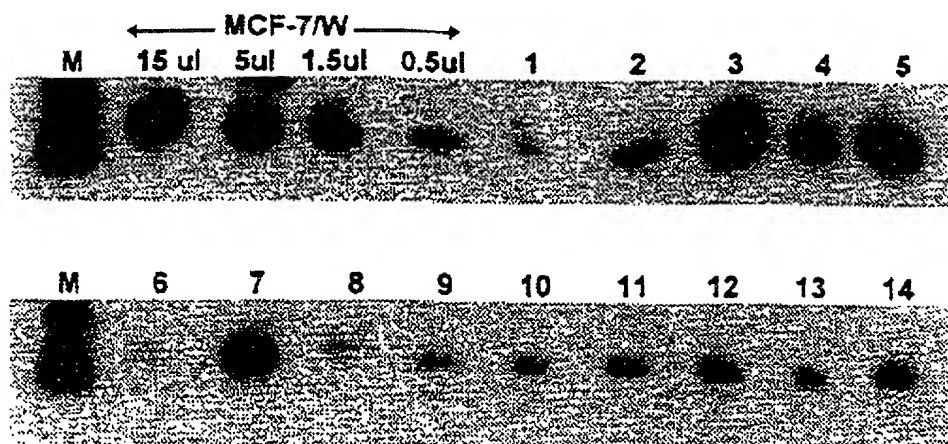




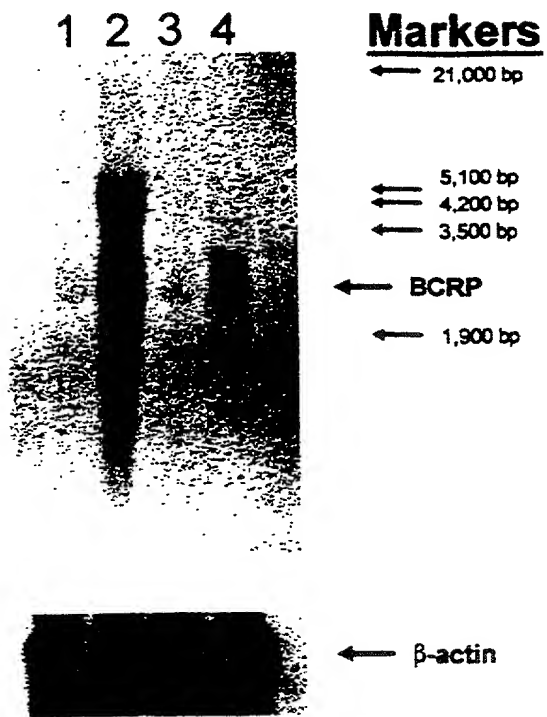
Figure 7.

**RT-PCR detection of BCRP mRNA expression in MCF-7/W cells  
or Blast Cells from Patients with Acute Myeloid Leukemia**



**Figure 7.** Detection of the expression of BCRP mRNA transcripts in MCF-7/W cells or in blast cells from 14 patients with AML. Total cellular RNA was isolated as described previously (10), then 1 ug of RNA was added to a reverse-translation reaction mixture containing AMV reverse transcriptase, and oligonucleotide primers specific for beta-actin (10) or BCRP (see Example 11), as described previously (10). Following reverse-transcription, PCR was performed as described in Example 11, then an aliquot of the PCR reaction mix was subjected to agarose gel electrophoresis. For BCRP, the agarose gel was transferred to nitrocellulose membranes then Southern hybridization was done using <sup>32</sup>P-labeled "clone-8 PCR product" as a probe for BCRP. A radioautograph of this Southern blot is shown in this figure. M=DNA size marker. The number under MCF-7 indicates the ul of PCR reaction mixture that was added to the agarose gel lane. The numbers 1 to 14 indicate an AML patient blast cell sample; 15 ul of PCR reaction mix were added per gel lane for the AML samples. For beta actin, the PCR product on ethidium-bromide stained gels was approximately equal for the patient samples and for an equivalent amount of MCF-7/W PCR reaction mixture (data not shown).

FIGURE 8A



**FIGURE 8B**

1 2 3 4 5 6 7 8 9 10

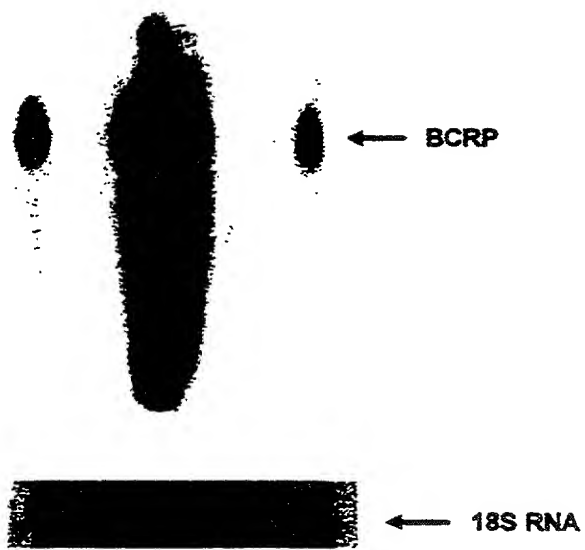


FIGURE 8C

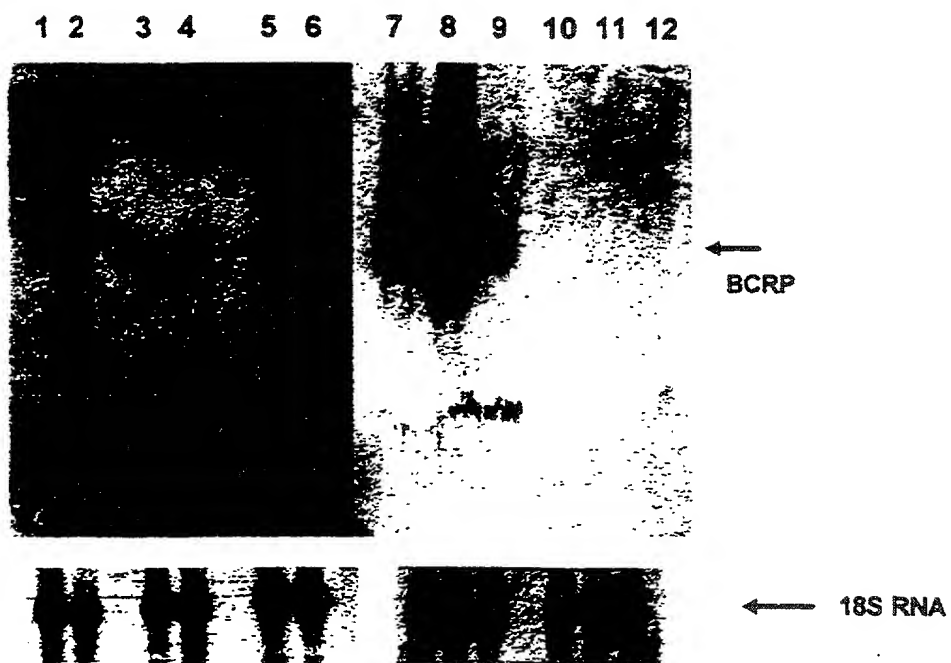


FIGURE 9

